Bepaling in vitro van de levensduur en de resistentie van erythrocyten bij de mens

Heerspink, Willem

SUMMARY

Chapter I and II

After some introductory remarks on the erythrocyte's normal course of life and some criticisms concerning current terminology, a review is presented of the literature on methods for the determination of resistance and survival time of erythrocytes.

The survey of the resistance techniques is divided in three parts. The first part describes the osmotic resistance method with some of the variations in procedure, the second the mechanical resistance method. In the third part a detailed review of the pH-resistance method is presented. This method, which has been proven to be more valuable than the osmotic and mechanical resistance techniques, is a very important aid in the investigation of a number of haematological diseases. In many cases, in which the osmotic resistance is still normal, the pH-resistance already shows an aberrant pattern.

The development of methods for the evalution of erythrocyte life span is given next. Special attention is paid to the differential agglutination technique according to Ashby and to the tracer technique of Gray and Sterling[30], depending on labelling erythrocytes with radioactive chromium. The hyperbolic shape of the survival curve, was found using the isotope technique and the usual explanation of it (elution theory) were among the reasons behind this thesis.

The experimental investigation included the study of the resistance of erythrocytes, using the pH-resistance technique, and the study of the erythrocyte survival time using the ^{51}Cr labelling method.

Chapter III

The influence of the pH on the haemolysis of erythrocytes, as described by van Kampen et al.[45] in 1954, was re-investigated. In

normal erythrocytes haemolysis begins at pH 5.3. In haemolytic disease, haemolysis starts earlier i.e. at higher pH values. The degree of haemolysis was measured photometrically, using the hemiglobincyanide method. In nearly all patients with haemolytic disease decreased pH-resistance was found. Since even slight disturbances may be detected this method appears to be of real diagnostic value.

The haemolysis of erythrocytes in alkaline buffersolutions was also studied. This, however, was discontinued because of the poor reproducibility.

Next the pH influence on the ^{51}Cr-uptake by erythrocytes was studied. In the physiological pH range there proved to be a strong dependence on the hydrogen ion concentration, the uptake varying with a factor 2 to 3 for a pH-change of about one unit.

Chapter IV

The estimation *in vivo* of the apparent half survival time with ^{51}Cr labelled erythrocytes is described first.

The uptake *in vitro* of ^{51}Cr by erythrocytes was studied. This uptake may be measured when whole blood is incubated at 37° C. with a $Na_2{}^{51}CrO_4$ solution. The total radioactivity is measured first, then the radioactivity of the centrifuged erythrocytes is determined. From these data the percentage of ^{51}Cr taken up may be calculated. The influence of the number of erythrocytes in the sample and the amount of ^{51}Cr was investigated. Fig. 4 shows the correlation between the ^{51}Cr uptake in percents and the number of erythrocytes per microliter.

It was found that erythrocytes from patients with haematological disorders bind more ^{51}Cr than normal erythrocytes (fig. 5). The conclusion was drawn that the older the erythrocyte, the more ^{51}Cr is taken up. It is known that the life span of the red cells is diminished in haematological disorders which might explain the increased binding of ^{51}Cr in the *in vitro* technique.

It is noteworthing that the *percentage* ^{51}Cr uptake is not influenced by the concentration of the chromate solution, while the *absolute* quantity of ^{51}Cr taken up by the red cells is different. This problem is discussed in detail in chapter VI.

The concept of the Q-index, meaning the quotient of percentage ^{51}Cr uptake of a blood sample and the normal uptake, in relation

to the same number of erythrocytes, is introduced. This Q-index appeared to be related to the apparent half survival time estimated *in vivo* using radioactive chromium. This relationship is linear (fig. 6). It is therefore possible to calculate the apparent half survival time from the ^{51}Cr uptake *in vitro*. This method can replace the time consuming *in vivo* technique. The patient is not exposed to radiation and the results are known within some hours.

Chapter V

Evidence is presented that the older erythrocytes take up the most chromium while the young cells hardly take up any. Mollison's elution theory is therefore not necessary to explain the hyperbolic shape of the erythrocyte survival curve.

It is possible to divide an erythrocyte population into different age groups using hypotonic saline solutions. The oldest cells haemolyse first, while the youngest cells are the most resistant. Buffer-solutions of different pH may also be used to divide an erythrocyte population into age groups. It was found that, using different age groups, the oldest cells bind the most ^{51}Cr, which is in accordance with the hypothesis described in chapter IV.

The investigation was repeated in a reversed order. The erythrocytes were labelled first with ^{51}Cr and then haemolysed. It was again found that the ^{51}Cr uptake was a function of the age of the erythrocyte.

For a number of patients with a low erythrocyte count, suffering from pernicious anaemia, the Q-index was determined. After vitamine B_{12} therapy the reticulocyte count was raised, which means that in the erythrocyte population there was an increase of young cells. The Q-index was determined for some weeks. It was found that the Q-index decreased with increasing reticulocyte count, even to subnormal values, finally becoming normal. This observation is the most convincing demonstration that senescent cells take up the most chromium. The elution theory as an explanation for the shape of the erythrocyte survival curve can therefore be discarded.

Chapter VI

Some theoretical considerations on the experimental results are given. It was found that the uptake of CrO_4^{--}ions by erythrocytes

may be, in first approximation, represented by Freundlich's equation:

$$\frac{x}{m} = k\, c_p^n \tag{1}$$

where x/m represents the absolute amount of chromium taken up by 10^6 erythrocytes, c_p the ^{51}Cr-concentration in the plasma after incubation and where n is a constant.

However it was also found that the value of k was related to the ^{51}Cr-concentration of the stocksolution used. This relationship proved exponential and could be represented by the following equation.

$$k = p\, c_s^q \tag{3}$$

where p and q are constants and c_s represents the ^{51}Cr concentration of the stock solution. Through substitution of (3) in (1):

$$\frac{x}{m} = p\, c_s^q\, c_p^n \tag{5}$$

This equation is an expansion of Freundlich's formula. The first phase of the ^{51}Cr uptake may therefore be thought to be an adsorption process. The dependence of k on the ^{51}Cr concentration indicates that the process of chromium uptake is not adsorption only.

Finally a practical equation is derived, introducing the desintegration time of ^{51}Cr in equation (5), supposing the concentration of ^{51}Cr at time zero to be 100 μC per ml. Using this equation (see equation (6) p. 52) the amount of ^{51}Cr in the erythrocytes, in correlation to the ^{51}Cr plasma concentration, may be calculated at any given moment.

Chapter VII

In this chapter some factors influencing the ageing process of erythrocytes are discussed. Enzyme concentration changes, especially of glucose-6-phosphate dehydrogenase, are reviewed. Also discussed are the time-dependent changes in the structure of haemoglobin. The conclusion is drawn that a gradual change in erythrocyte metabolism, resulting in changes of the membrane, are the cause of a slowly developing change in the permeability. This change in permeability explains the increased ^{51}Cr adsorption, thus higher uptake of the label. The ^{51}Cr uptake is possible first an adsorption process as described by Freundlich, followed by a second, as yet unknown, stage.